REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE From June 91-May 96

3. REPORT TYPE AND DATES COVERED FINAL REPORT

4. TITLE AND SUBTITLE Characterization of selected bacteria and enzymes involved in the sequential anaerobic degradation of 2,4-dichlorophenol

5. FUNDING NUMBERS N00014-91-J-1874

6. AUTHOR(S)

Juergen Wiegel

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Georgia
Department of Microbiology
Athens, GA 30602-2605

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5660

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Unlimited

19970318 138

13. ABSTRACT *(Maximum 200 words)*

We elucidated the pathway for the anaerobic degradation of chlorophenols under methanogenic conditions and studied the microbial interactions of the community being directly or indirectly involved in this process. At least 6 different bacteria, --constituting a sequential pathway--, are required. We studied the influence of various environmental factors on the degradation rates in sediment samples and directly in the environment. During the grant period, we isolated two of the main members of this pathway: 1) Desulfitobacterium dehalogenans gen. nov., sp. nov., which catalyzes specifically the removal of ortho substituted phenolic chlorines. An extensive substrate specificity and structure-function analysis, revealed that especially the *para* position of the halophenols can be substituted with a great variety of groups including carboxylic-, nitro-, amino-, methyl-, hydroxyl-, halogen-, and aryl-substituents leading to a wide variety of different compound classes. We started on the purification of the dehalogenase. 2) *Clostridium hydroxybenzoicum* sp. nov., which catalyzes the important step of forming hydroxybenzoate and thus linking in the degradation chain the dehalogenation and the mineralization of the dehalogenated phenolic compounds. The organism harbors two specifically induced reversible hydroxybenzoate decarboxylases, which we purified and characterized. Most of the work has been published in various international journals.

14. SUBJECT TERMS Anaerobic degradation, reductive dehalogenation, enzyme purification, isolation/characterization of anaerobic bacteria, aryldehalogenation, aryldecarboxylases

15. NUMBER OF PAGES 6

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
U	U	U	U

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."
DOE - See authorities.
NASA - See Handbook NHB 2200.2.
NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.
DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
NASA - Leave blank.
NTIS - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FINAL REPORT

GRANTt #: N00014-91-J-1874 R&T Code: 4412211
(including a 1 yr limited and 1 yr no-cost extension)

PRINCIPAL INVESTIGATOR: Juergen Wiegel

INSTITUTION: University of Georgia

GRANT TITLE: Characterization of selected bacteria and enzymes involved in the sequential, anaerobic degradation of 2,4-dichlorophenol

REPORTING PERIOD: 1 June -1991 - 31 May 1996

STATEMENT OF OBJECTIVES (from Original Grant)
LONG TERM GOAL: Understanding the complexity of microbial communities involved in bioremediation. This means understanding the microbial interactions and the biochemical principles involved in anaerobic degradation of chlorinated xenobiotics in marine and fresh water sediments. This includes answering the question of whether there are and if, what are the basic differences in the degradative principles observed in marine and freshwater communities involved in the degradation of chlorinated xenobiotics. We will compare structures of isolated consortia and compare microbial interactions of their key players. Seeking an understanding also includes purifying, characterizing and sequencing key enzymes involved in the sequential degradation of our model compounds.

SHORT TERM GOALS:
From Original Grant: Isolation of the anaerobic 2,4-dichlorophenol-dehalogenating organism and purification of the 2,4-dichlorophenol dehalogenase either from the isolate or the existing 3-member mixed culture. Also elucidation of the transformation mechanism of phenol to benzoate including the purification of two newly identified aryl decarboxylases converting specifically hydroxybenzoates to phenol
From the 1-year limited Continuation Grant: .This grant is to obtain support for the education of two graduate students in the field of environmental microbiology and biochemistry. At the same time we will obtain important data for the above stated goal.

APPROACH: Mainly sediments from a 'pristine' freshwater lake at Sandy Creek Nature Park in Athens, GA exhibiting the desired dehalogenation and mineralization activities were chosen for the studies. A highly enriched 2,4-dichlorophenol dehalogenating enrichment had previously been obtained. Sediments were incubated under various conditions and analyzed for dehalogenation and degradation with the goal to elucidate dependencies between the different involved microorganisms as outlined previously (Zhang and Wiegel. Appl. Environ. Microbiol. 56:1119-1127 (1990)). Using a great variety of approaches (including soft agar-shake-gels)we isolated both the 2,4-dichlorophenol dehalogenating bacterium and the bacterium which is involved in the conversion of phenol to benzoate via the 4-hydroxybenzoate intermediate. From finally isolated pure microorganisms the enzymes were purified using traditional biochemical methods but performing all purification steps in an anaerobic chamber located in a cold room. The latter was necessary since the enzymes are highly oxygen-labile and instable at room temperatures. Substrate analogues were used as additives to the buffer to obtain a stabilization by substrate(analogue)-enzyme complex formation.

ACCOMPLISHMENTS:

Isolation and characterization of novel bacteria involved in the anaerobic degradation of chlorophenols under methanogenic conditions: From the previously published proposed substrate-product-substrate pathway, we were able to isolate two of the key microorganisms. Both are novel bacteria: *Desulfitobacterium dehalogenans* gen. nov. sp. nov., the first in the pathway, which dehalogenates 2,4-dichlorophenol to 4-chlorophenol and *Clostridium hydroxybenzoicum* sp. nov, which converts the dehalogenated phenol to the proposed important intermediate 4-hydroxybenzoate. Both bacteria were characterized in respect to phylogeny and taxonomy (literature cited below: A.3; A.4) and in respect to their substrate specificity (A.5; A.6;)

The most striking property of *C. hydroxybenzoicum* is that it has two specifically induced enzymes for decarboxylation of 4-hydroxybenzoate and for 3,4-dihydroxybenzoate, respectively. The bacterium, however, can not further metabolize either the phenol or the hydroxybenzoate. Thus, the true function of this enzyme in this and similar (B.12) bacteria has still to be regarded as unknown. However, using 4-hydroxybenzoate as supplement to the medium, the organism was enriched in the 2,4-dichlorophenol degrading enrichment. A remarkable anctivity of 100 mmol hydroxybenzoate/l decarboxylated per 24 h was finally reached. It is speculated that the stimulating acetate produced from benzoate is the reason for this high enrichment in a 2,4-dichlorophenol degrading cultures.

D. dehalogenans was the first validly described species from a group of presently three other dehalogenating species which subsequently were isolated and described by other laboratories. Our organism and some of the other species can also grow using Fe(III) as electron donor and produce the reduced Fe(II)-containing magnetic mineral, magnetite. *D. dehalogenans* exhibited in respect to the stereospecificity of the removable halogen a high specificity. It dehalogenated only halogens (chlorines, bromides) substituted in the phenolic *ortho* position. However, in respect to further substitution, the dehalogenation reaction exhibited a surprisingly wide substrate spectrum. Many different classes of compounds (aryl acids, nitroaromatic compounds, aminophenols etc.) were dehalogenated as long as the chlorine is in *ortho* position to an aromatic hydroxyl group. These compounds included pentachlorophenol (A.6) and compounds which had many different substitutions in the 4 position to the hydroxyl group; the substitution included even a second substituted phenyl ring. So far, there is no other anaerobic bacterium known which can dehalogenate hydroxylated PCBs (ms in preparation). *D. dehalogenans* can also utilize as electron donors hydrogen or organic substrates such as pyruvate. We have demonstrated that the dehalogenation reaction is used just as an alternate electron accepting reaction and that it leads to energy formation assumingly via electron transport phosphorylation (C. 4; D. 14; attached ms-draft).

We have not succeeded (to our knowledge, nor any one else) to isolate the 4-chlorophenol dehalogenating microorganism. However, we were able to maintain the activity in a rotary felt disc fermentor (increased surface) under continous conditions. This is a first step to a possible succesful isolation.

Microbial interaction of anaerobic bacteria in sediments and cultures degrading chlorophenols

In several papers we elucidated the environmental parameters governing the rate of dehalogenation in the environment (A.1; A.2, B.9-B13 and in preparation). The results indicate that the first step in the degradation chain can be influenced not only by various environmental factors acting directly on the dehalogenating bacterium(bacteria) but also by the influence on various non-dehalogenating microorganisms in the community which produce or compete through their metabolism and thus influenced the availability of electron donors or other electron acceptors to the dehalogenating microorganism(s). The chimeric compound, 3-chloro-4-hydroxybenzoate (functioning as either a hydroxybenzoate, 2-chlorophenol, 3-chlorobenzoate, 2Cl-4OH-benzoate) was used to demonstrate that the degradation route was dependent on the history of the sediment. The presence of either hydroxybenzoate (which induced decarboxylation) and 2-chlorphenol or 2,4-dichlorophenol .(which

induced dehalogenation) determined whether first a dehalogenation or a decarboxylation occurred.. . In all cases, phenol was the intermediate which then was again carboxylated and metabolized via benzoate to methane and carbon dioxide. This serves as an example of the more complex degradation pathways for such compounds under anaerobic (methanogenic) conditions in comparison to strict aerobic pathways. This does not mean that the degradation is less efficient. The required cooperation even can be an advantage.

Purification of key enzymes:

1) ***Ortho*-chlorophenol dehalogenase:** We developed an assay for the dehalogenase (removing *ortho* chlorines from substituted phenols) using mainly 3-Cl-4-OH-benzoate(which exhibited one of the highest activities but little toxicity at elevated concentrations) as the e-acceptor and pyruvate as e-donor. We determined that 3/4 of the total enzyme activity is associated with the membranes whereas the rest is in the cytoplasm. Glycerol and Triton X-100 solubilized the enzyme but not CHAPS or high salt concentrations The latter fact indicates that the enzyme is an integral part of the membrane. The enzyme is not extremely oxygen sensitive. We have started with the further purification using chromatographic columns for separation, however, we had to stop this work due to lacks of funds before the enzyme was purified. Fractions of partially purified enzyme are frozen away at minus 70°C.

2) Reversible hydroxybenzoate decarboxylases: Both the 4-hydroxybenzoate decarboxylase and the 3,4-dihydroxybenzoate decarboxylase are novel enzymes and can function in both directions, i.e., carboxylate phenols without the presence of biotin, (not inhibited by avidin), thiamin pyrophosphate or pyridoxal5'-phosphate (no inhibition by hydroxylamine), or the requirement of ATP (A.5; A.7; A.8) or decarboxylate hydroxybenzoates. We ensured the reversibility of both purified enzymes with gel electrophoretic pure enzyme preparations and determined the equilibrium, of the reaction. For both enzymes, as expected from the whole cell experiments (A 5), the equilibrium is far on the side of the phenolic compounds ((A.7; A.8). Both enzymes --purified to SDS-gel electrophoresis homogeneity --are extremely oxygen sensitive and thus were purified using an anaerobic chamber in a cold room. Both enzymes appear relatively similar in subunit size but have slightly different N-terminal sequences. The 4-hydroxybenzoate decarboxylase apparently a hexamer whereas the other is a pentamer . We have started to clone the genes for these enzymes using probes from internally derived amino acid sequences since the N-terminal sequences were toounspecific (presently on halt due to missing grant support). Further, we have studied the reaction mechanism using deuterated substrate and chemical modification techniques (Z. He, Ph. Dissertation, ms in prep.).

Students Trained:

Ilya Utkin (postdoctoral candidate; 1992-1995); *Xiaming Zhang* (Ph.D. 1993); *Kuo-Chou Chuang* (MS, 1995), *Qingzhong Wu* (Ph.D. 1996), *Peter Knoblich* (Diplom Univ.Karlsruhe, 1996); *Mark Mackiewicz* (MS, 1997) *Rongdi Shan* (Undergraduate 1993/94)

Publications on the degradation of 2,4-DCP and related compounds which resulted directly or indirectly from the Navy Grant.

From the originally anticipated five manuscripts mentioned in the grant application, three of them were published as anticipated, however, one (survey on the distribution of the special decarboxylases) is still under preparation, and the other one (purification of the dehalogenase) can not yet be written since the enzyme is not yet pure (master thesis from M. Mackiewicz). But due significant overall progress in the elucidationof the chlorophenol degradation under anarobic conditions, a total of 8 publication in highly respected peer-reviewed journals plus 4 book chapters/review articles were published (totalling 12 publication supported by this ONR-grant; see list below). In addition, 2 Ph.D. dissertations and two MS-theses were published on the ONR-supported work. Furthermore, 4 additional papers describing ONR supported work are expected to follow this or early next year.

A. Original research papers in peer-reviewed journals:

1. D. D. Hale, J. E. Rogers and J. Wiegel. 1991. Environmental factors correlating to dichlorophenol dechlorination in anoxic freshwater sediments. Environ. Toxicol. Chem. *10*:1255-1265. (ONR funded my time for writing the ms)
2. X. Zhang and J. Wiegel. (1992). The anaerobic degradation of 3-chloro-4 hydroxybenzoate in freshwater sediments proceeds either via chlorophenol or hydroxybenzoate to phenol before it is carboxylated to benzoate. Appl. Environ. Microbial. 58:3580-3585.
3. X. Zhang, L. Mandelco, and J. Wiegel. (1994) *Clostridium hydroxobenzoicum* sp. nov.: an amino acid utilizing, hydroxybenzoate decarboxylating bacterium isolated from a methanogenic freshwater pond sediment. Int. J. Syst. Bacteriol. 44:214-222
4. I. Utkin, C. Woese, and J. Wiegel. 1994 Isolation and characterization of *Desulfitobacterium dehalogenans* gen. nov. sp. nov., an anaerobic bacterium which reductively dechlorinates chlorophenolic compounds. Int. J. Syst. Bacteriol. 44: 612-619.
5. X. Zhang and J. Wiegel. 1994. Reversible conversion of 4-hydroxybenzoate and phenol by *Clostridium hydroxybenzoicum*. Appl.. Environ. Microbial. 60:4182-4185.
6. I. Utkin, D. D. Dalton and J. Wiegel. 1995. Specificity of reductive dechlorination of substituted *ortho*-chlorophenols by *Desulfitobacterium dehalogenans* JW/IU-DC1. Appl.. Environ. Microbial. 61:346-351.
7. Z. He and J. Wiegel. 1995. Purification and characterization of an oxygen-sensitive reversible 4-hydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum* Eur. J. Biochem. 229: 77-82
8. Z. He, and J. Wiegel. 1996. Purification and Characterization of an oxygen-sensitive, reversible 3,4-dihydroxybenzoate decarboxylase from *Clostridium hydroxybenzoicum*. Bacteriol. 178:3539-3543

4 more papers are still in preparation.

A total of 28 peer-reviewed, orignal research manuscripts were published by the PI within the time covered by this report.

B. Book Chapters and Reviews

9. Wiegel, J., G.-W. Kohring, X. Zhang, I Utkin, D. Dalton, Z. He, Q. Wu, and D. L. Bedard. 1992. Temperature, an important factor in the anaerobic transformation and degradation of chlorophenols and PCBs. In: Soil Decontamination Using Biological Processes. (Proceeding Int. Symposium, Karlsruhe, 1992), pp. 101-108. DECHEMA, Frankfurt/Main.
10 Wu, Q., D. L. Bedard and J. Wiegel. 1992. Effect of temperature on the dechlorination of PCBs in methanogenic freshwater sediments. In: Soil Decontamination Using Biological Processes. (Proceeding Int. Symposium, Karlsruhe, 1992), pp. 491-497.
11. D. D. Hale, J. E. Rogers and J. Wiegel. 1992. Reductive dechlorination of dichlorophenols in anaerobic pond sediments. IN Organic substances and sediments water. Vol. III (R.A. Baker, ed.)Chapter 13; pp.211-222, Lewis Publishers, Inc. Boca Raton, FL.

12. Zhang, X., R. L. Gherna and J. Wiegel. 1992. Decarboxylation of hydroxy benzoates by strictly anaerobic bacteria in methanogenic sediments and pure cultures. In: Soil Decontamination Using Biological Processes. (Proceeding Int. Symposium, Karlsruhe, 1992), pp. 498-503.

13. D. D. Hale, W. Reineke and J. Wiegel. Chlorophenol degradation. In: Biological degradation and bioremediation technologies of toxic chemicals (G.R. Chaudry, Ed.), Chapter 4, pp. 74-91. Timber Press, Portland, OR.

A total of 11 bookchapters and reviews were published within the time covered by this report.

C. Dissertations and MS-theses:

1. X. Zhang. Sequential degradation of chlorophenols in anaerobic freshwater sediments. Ph.D. dissertation Univ. of Georgia, Athens GA. 1993
2. Z. He. Reversible hydroxybenzoate decarboxylases from the anaerobic bacterium *Clostridium hydroxybenzoicum*. Ph.D. dissertation Univ. of Georgia, Athens GA. 1996
3. P. Knoblich. Growth of Desulfitobacterium dehalogenans, an anaerobic bacterium capable of reductive dehalogenation, in continuous culture with increased surface area. Diplom Arbeit fuer Univ, Karlsruhe. Experimental work performed at the University of Georgia, Athens GA. 1996
4. M. Mackiewicz. Growth yields and electron acceptor specificity of *Desulfitobacterium dehalogenans* and purification of the ortho chlorophenol dehalogenase. MS.thesis Univ. of Georgia, Athens GA. May 1997 (in preparation)

D. Poster presentations by student/postdoctoral candidates supported by ONR:

1. I. Utkin and J. Wiegel. Anaerobic dehalogenation of chlorophenols and its analogues. 2nd Annual University System of Georgia Research Symposium, Athens, GA, May 1992. Abstr. Session 2, 230.
2. I. Utkin and J. Wiegel. Anaerobic dehalogenation of chlorophenols and their analogs. ASM-Conference Anaerobic Dehalogenation and its Environmental Implications. Athens, GA, 1992, Abstr. #23.
3. X. Zhang, Z. He and J. Wiegel. Effect of added nutrients on anaerobic transformation of chlorinated compounds. ASM-Conference Anaerobic Dehalogenation and its Environmental Implications. Athens, GA, 1992. Abstr. #24.
4. , K. S. Chuang, D. Bedard, and J. Wiegel. Effects of temperature on the anaerobic transformation of PCBs in Woods Pond sediment slurries. ASM-Conference Anaerobic Dehalogenation and its Environmental Implications. Athens, GA, 1992. Abstr. #25.
5. I. B. Utkin and J. Wiegel. Nutritional requirements for reductive *ortho*-dehalogenation of chlorophenolic compounds. Ann. Meet. Am. Soc. Microbial. Atlanta, May 1993.
6. K. S. Chuang, Q. Wu, D. L. Bedard, and J. Wiegel. Effect of pH and temperatures on the dechlorination of polychlorinated biphenyl (PCB) in Woods Pond sediments. Ann. Meet. Am. Soc. Microbial. Atlanta, May 1993.
7. Q. Wu and J. Wiegel. Effect of partial H_2 pressure on the reductive dechlorination of polychlorinated biphenyls in Woods Pond sediment. Ann. Meet. Am. Soc. Microbial., Las Vegas, May 1994. Abstr. Q322.
8. Z. He and J. Wiegel. Characterization of purified 4-hydroxybenzoate and 3,4-dihydroxybenzoate decarboxylase (carboxylases) from *Clostridium hydroxybenzoicum*. Ann. Meet. Am. Soc. Microbial. Las Vegas, May 1994. Abstr. Q458.
9. I. Utkin, C. Woese, and J. Wiegel. Reductive dechlorination of chlorophenols by *Desulfitobacterium dehalogenans*, gen. nov., sp. nov. IUMS 7th Intern. Congress of Bacteriology and Applied Microbiology Division, Prague July 1994. Abstr.
10. J. Wiegel, Z. He, and X. Zhang. Carboxylation and decarboxylation of hydroxylated aromatic compounds by anaerobic bacteria (inv. presentation) 3rd. Intern. Conf. on Carbon Dioxide Utilization (ICCDU III) Norman OK, April 1995 (Oral Communication Session Abstracts)
11. Q. Wu, K.S. Chuang, and J. Wiegel. Anaerobic enrichment cultures capable of dechlorinating lonely *para*-chlorine-substituted polychlorinated biphenyl congeners. Ann. Mee. Am. Soc. Microbial. Washington DC, May 1995 Abstr. Q90
12. J. Brill, P. Youngman, and J. Wiegel. Detection of sporulation-specific genes in thermophilic, nonsporeforming isolates. Ann. Mee. Am. Soc. Microbial. Washington DC, May 1995. Abstr. R32

13. M. Mackiewicz and J. Wiegel Comparison of energy and growth yields for Desulfitobacterium dehalogenans when grown in the presence of various terminal electron acceptors. Ann. Mee. Am. Soc. Microbial. New Orleans, May 19996, Q-175
14. Q, Wu and J. Wiegel Effects of temperature shift on the reductive dechlorination of polychlorinated biphenyls in Woods Pond sediments. Ann. Mee. Am. Soc. Microbial. New Orleans, May 19996, Q-198
15. J. Brill and J. Wiegel. Sporulation genes in non sporulating bacteria and sequence of a small acid soluble protein (SASP). A sporulation protein in the non spore former Thermoanaerobacter ethanolicus. Thermophiles '96, Intern. Conference Athens, GA. Sept. 96. (Abstr. S.B.7)
16. J. Wiegel: Reductive dehalogenation of halogenated aromatic compounds. Internat. Meet. "The Art of Anaerobes", Athens, GA, Aug. 96.

Total of 33 poster presentation were made during the reporting time by the investigators research group.

E. Invited lectures and Seminars on ONR-supported Research:

1. Anaerobic dechlorination of haloaromatic compounds. University of Waikato, September 1991.
2. Anaerobic degradation of chlorinated aromatic compounds. University of Christ Church, South Island New Zealand, September 1991.
3. Anaerobic degradation of chlorinated aromatic compounds. University of Bangkok (Thailand) September 1991.
4. Anaerobic degradation of chlorinated aromatic compounds. University of Regensburg (Germany, October 1991).
5. Reductive dechlorination and the fate of 3-Cl-4-OH benzoate in freshwater sediments. University of Wageningen, (The Netherlands), December 1991.
6. Anaerobic degradation of chlorinated aromatic compounds. Department of Biochemistry and Center for Metalloenzymes Studies, University of Georgia, March 1992.
7. Anaerobic dechlorination. DuPont, Wilmington, DL. June 1992.
9. Sequential degradation of 2,4-dichlorophenol. ASM-Conference on Anaerobic Dehalogenation and its Environmental Implications. Athens, September 1992, (invited lecture).
10. Effect of temperature on the dechlorination of PCB's in Woods Pond Sediment. ASM-Conference on Anaerobic Dehalogenation and its Environmental Implications. Athens, September 1992 (invited lecture).
11. PCB-degradation under anaerobic conditions. Department of Microbiology, University of Saarbrucken, Saarbrucken, Germany, December 1992.
12. Temperature - an important factor in the anaerobic transformation and degradation of chlorophenols and PCBs. Dechema-Symposium: Soil Decontamination Using Biological Processes, Karlsruhe, December 1992, (invited plenum talk).
13. Effect on environmental parameters on the anaerobic transformation of PCBs and PCB-congeners in contaminated freshwater sediments. ACS Meeting Emerging technologies in Hazardous Waste Management V. September 1993 (invited seminar talk).
14. Anaerobic degradation of PCB. University of Bergen, Norway, October 1993 (invited seminar).
15. Anaerobic reductive dechlorination of aromatic compounds (inv. seminar at 60th birthday of Prof. Gottschalk) April, 1995
16. The novel group of extremely fast growing alkalithermophiles. Seminar at the Von Humboldt Univ. Berlin , Germany. Juli 1995
17. Diversity and Distribution of *Thermoanaerobacterium* species (Inv. Lecture at 1st Yellowstone Nat.Park Symposium on Biodiversity, Ecology, and Evolution of Thermophiles in Yellowstone National Park: Overview and Issues. Old Faithful, Aug. 1995
18. Effect of environmental parameters on reductive dechlorination of PCBs and chlorophenols. (inv. lecture) IBC's Intern. Symposium on Biological Dehalogenation, Annapolis, MD. Oct. 1995
19. Reductive dehalogenation. Univ. of Occupational Health (Seminar), Kita-Kushu, Japan; July 1996

A total of 35 invited seminars and lectures were given by the investigator during the funding period.